Title: Moderate N fertilizer reduction with straw return modulates ecosystem services and microbial traits in a meadow soil

删除[DY]: **Straw return with diverse nitrogen fertilizer application rates modulate ecosystem services and microbial traits in a meadow soil**

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Abstract:

Nitrogen (N) fertilization has received worldwide attention due to its benefits to soil fertility and productivity, but excess N application also causes an array of ecosystem dis-services, such as greenhouse gas emissions. Generally, soil microorganisms are considered to be involved in upholding a variety of ecosystem services and dis-services. However, the linkages between soil ecosystem services and microbial traits under different N fertilizer application rates remain uncertain. To address this, a 4-year in situ field experiment was conducted in a meadow soil on the Northeast China Plain after straw return with the following treatments combined with regular phosphorus (P) and potassium (K) fertilization: (i) regular N fertilizer (N+PK); (ii) 25% N fertilizer reduction (0.75N+PK); (iii) 50% N fertilizer reduction (0.5N+PK); and (IV) no N fertilizer (PK). Ecosystem services, dis-services and microbial traits responded distinctly to the different N fertilizer rates. Treatment 0.75N+PK had overall positive effects on soil fertility, productivity, straw decomposition, and microbial abundance and function and alleviated greenhouse effects. Specifically, no significant difference was observed in SOC, total N, P content, straw C, N release amounts, microbial biomass C, N content, as well as celluase and N-acetyl-D-glucosamindase activities, which were all significantly higher than 0.5N+PK and PK. Greenhouse gas mineralization was reduced with the decresing of N input levels. Moreover, the highest straw biomass and yield were measured in 0.75N+PK, which were significantly higher than 0.5N+PK and PK. Meanwhile, 0.75N+PK upregulated aboveground biomass and soil C:N and thus increased the abundance of genes encoding cellulose-degrading enzymes, which may imply the potential ability of C and N turnover. In addition, most observed changes in ecosystem services and dis-services were strongly associated with microbial modules and keystone taxa. The *Lasiosphaeriaceae*-driven module 1 community promoted straw degradation and C and N release, while the *Terrimonas*-driven module 3

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community contributed to production improvement, which was conducive to soil multifunctionality. Therefore, our results suggest that straw return with 25% chemical N fertilizer reduction is optimal for achieving ecosystem services. This study highlights the importance of abiotic and biotic factors in soil health and supports green agricultural development by optimizing N fertilizer rates in meadow soil after straw return.

Keywords: Ecosystem services; Straw return; Nitrogen fertilization; Microbial community; Crop yield

1. Introduction

Multiple soil ecosystem services are indicators of soil health (Kihara et al., 2020; Lehmann et al., 2021). Soil ecosystem services refer to the ability of soil to function as a vital living system to sustainably increase crop productivity, improve environmental quality, tackle climate change and promote plant and animal health (de Bello et al., 2010; Tang et al., 2019). Intensive agriculture has posed a wide range of threats to agroecosystem services (Robertson et al., 2014; Allen et al., 2015). Irrational application of chemical fertilizers, especially nitrogen (N), is ubiquitous to achieve high crop yields in response to population surges globally (Shi et al., 2019). In fact, N is considered the essential macronutrient for all biota, while excessive N fertilizer inputs not only reduce soil fertility and productivity but also lead to environmental burdens (Trost et al., 2016). Recent researched indicated that appropriate reduction of N fertilizer input can not only maintain crop yield by increasing N fertilizer use efficiency, but promote soil health by regulating soil C: N ratio (Chen et al., 2014). However, excessive reduction of N fertilizer input can lead to a “N-mining” effect, resulting in the loss of soil organic matter, which reduces crop yields (Chen et al., 2014). Therefore, how to achieve agroecosystem services by regulating N fertilizer application rates needs to be fully assessed.

Straw return has also been widely applied as a major measure to moderate soil ecosystem services

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删除[DY]: For example, previous studies emphasized that N fertilizer abuse may accelerate greenhouse gas emissions (Huang et al., 2006; Wu et al., 2015) and degrade groundwater quality (Rhymes et al., 2016).

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(Xu et al., 2021). Plant residues contain abundant N that further affects soil fertility and productivity (Pan et al., 2009; Liu et al., 2014). Thus, the straw-derived N released during degradation is an important source that may serve as a partial substitute for chemical N fertilizer application (Wang et al., 2017; Latifmanesh et al., 2020). However, crop fields suffering from abundant organic materials usually have low reutilization efficiency (Hou et al., 2020). Generally, the majority of N in straw is released into the atmosphere as oxynitride, such as nitrous oxide (N_2O) (Wang et al., 2019; Sun et al., 2021). Subsequent literature highlighted that straw return significantly elevates greenhouse gas emissions so that less than 15% of straw-derived N can be transformed into soil and become SOM (Yin et al., 2018; Wu et al., 2019). However, the potential for the partial substitution of straw for chemical N fertilizer application is still unclear.

Soil microorganisms were the drivers of soil ecosystem services (Handa et al., 2014; Wagg et al., 2014). Agronomic management for such "multifunctionality" has prompted research into the role that microbes play in providing desired rates of multiple ecosystem processes (Gong et al., 2020). Fertilization-induced changes in microbial communities and functions are fundamental to the regulation of a variety of ecosystem multifunctionalities, including SOM formation, greenhouse gas emissions, litter decomposition, and crop production (Dominati et al., 2014). For example, Ning et al. (2020) found that long-term manure increased the abundance of specific fungi, which was involved in yield improvment. Duan et al. (2021) indicated that adequate N input improved cellulose-degrading ability of bacteria. To date, we still lack empirical evidence of the linkages among N fertilizers, specific microbial communities or functions and multiple ecosystem services, and the diverse cropland services driven by complex microbial traits under different N fertilizer rates are seldom clarified.

Microorganisms contribute to ecosystem services by modulating microbial function, community

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composition and succession, which are influenced by different N fertilizer input levels (Bradford et al., 2014; Chen et al., 2019a). Generally, bacteria and fungi are the main drivers of straw labile and recalcitrant component decomposition, respectively (Frey et al., 2013; Ge et al., 2017; Hogberg et al., 2007). In addition, microbial module communities and keystone taxa have been used to provide satisfactory explanations for ecosystem services. Chen et al. (2019b) found that particular microbial modules participated in N and phosphorus (P) turnover in a Cambisol. Actinobacteria have been extensively studied and can be considered the main degraders of straw by secreting cellulase (Bao et al., 2021). C, N and P stoichiometry has profound impacts on microbial in vivo metabolism and ex vivo modification processes (Chen et al., 2016). Nevertheless, the knowledge of the microbial mechanisms that modulate ecosystem services in response to N fertilizer input levels are still rudimentary.

As an important grain-producing region, the Northeast China Plain contributes to more than 20% of the total grain yield in China (Li et al., 2017; Zhao et al., 2018). Here, a field experiment was conducted to reveal the influences of N input levels on soil ecosystem multifunctionality and associated microbial traits. In the present study, two hypotheses were tested: (i) soil ecosystem services and dis-services would show distinct responses to N fertilizer input levels, and (ii) the changes in cropland ecosystem services and dis-services would be linked to specific microbial traits. The purpose of this study is to optimize the N fertilizer application rate to achieve soil ecosystem multifunctionality and explore the potential microbial mechanism in Mollisol.

2. Materials and methods

2.1 Site description and sampling

A field experiment under contrasting inorganic N fertilizer input levels was established in 2018 in Wenchun town (44°59′61″ N, 129°59′18″ E), Mudanjiang city, Heilongjiang Province, Northeast China

删除[DY]: makes understanding the consequences of the changes in microbial traits crucial for determining

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删除[DY]: *Mortierella* has been proven to increase soil fertility and crop yield due in part to its strong C sequestration capacity (Ning et al., 2020). Notably, it is also well known that microbial traits are mediated by nutrient availability and stoichiometry (Chen et al., 2014).

删除[DY]: Multiple studies have indicated that soil C:N and N:P ratios are the key factors mediating microbial functions and soil health (Ning et al., 2020; Duan et al., 2021).

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Plain, which is an important grain-producing area. This region has a typical temperate continental monsoon climate with an average annual temperature of 4.3 °C and a mean annual precipitation of 579.7 mm. The soil is classified as a meadow soil according to US Soil Taxonomy (USST). The cropping system was continuous maize (*Zea mays* L.) monoculture. Four treatments received different N fertilizer input levels after straw return to the field for 4 years as follows: (1) regular chemical fertilization, N+PK (300 kg urea (N 46%) ha^{-1} yr^{-1}, 250 kg diammonium phosphate (P_2O_5 48%) ha^{-1} yr^{-1}, 150 kg potassium chloride (K_2O 50%) ha^{-1} yr^{-1}); (2) 25% reduction of N fertilizer, 0.75N+PK (225 kg urea ha^{-1} yr^{-1}, 250 kg diammonium phosphate ha^{-1} yr^{-1}, 150 kg potassium chloride ha^{-1} yr^{-1}); (3) 50% reduction of N fertilizer, 0.50N+PK (150 kg urea ha^{-1} yr^{-1}, 250 kg diammonium phosphate ha^{-1} yr^{-1}, 150 kg potassium chloride ha^{-1} yr^{-1}); and (4) no N fertilizer, PK (250 kg diammonium phosphate ha^{-1} yr^{-1}, 150 kg potassium chloride ha^{-1} yr^{-1}). All straw and chemical fertilizers were applied with shallow tillage to 20 cm. Straw was cut into pieces less than 5 cm and input after the harvest in October, while the chemical fertilizers were applied during ploughing in May of the next year. All other normal management practices were consistent among treatments during the experiment. Before the experiment, the initial soil contained 18.74 g kg^{-1} SOC, 1.03 g kg^{-1} total N and 0.54 g kg^{-1} total P with a pH of 7.37 (H_2O). The yield and some of the soil chemical properties under different bulk soil treatments during the experimental process are shown in Supplemental material Table S1.

Soils were sampled after the maize harvest in October 2021. A randomized complete block design consisting of 4 treatments with 3 replications was adopted in this study. Each field plot was 4.5 m × 15 m. We took nine soil cores (5 cm diameter) from the top 20 cm of bulk soil in each plot. Each soil sample consisted of a mixture of subsamples randomly collected at nine different positions in the same plot. In total, 12 soil samples were collected from 4 treatments. Each treatment contained 3 replicates.

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Soils were sieved through a 2 mm mesh, the mineral particles and plant roots were carefully removed, and then the soils were homogenized and stored in an incubator at 4 °C in a 40% moisture environment. One part of the bulk soil sample was air-dried to measure basal soil properties, and the other part was used for microbial molecular analysis.

2.2 The field straw decomposition and carbon and nitrogen release experiments

The ditch-buried straw decomposition experiment was conducted using litter nylon bags. Maize straw materials were collected after maize harvesting in 2020 and air-dried. Ten grams of maize straw was cut to 2 cm in length and put into nylon litter bags, which were then sealed via heat sealing. The nylon bags were 6 cm × 10 cm in size and were made of 200 mesh nylon fabric, which permitted the free transfer of microorganisms between the nylon bags and soil. At 2ed, May 2021, litter bags containing straw were buried at 10 cm depth in a spatially random design to prevent bags associated with a given decomposition stage being placed together in space. The litter bags were collected after the harvest at 1st, October 2021.

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The straw decomposition ratio was calculated based on dry weight loss as (dry initial mass - dry final mass)/dry initial mass. The straw-C concentration was measured by titrimetry after oxidation with a mixture of H_2SO_4 and $K_2Cr_2O_7$. Total N, P and K were determined using the Kjeldahl, molybdenum blue colorimetry, and flame photometry methods, respectively. All methods have been described by Lu (2000). The initial and sampled maize straw material properties are shown in Supplemental material Table S2. The amounts of total straw C and N released were calculated by the following equation:

The amounts of total straw C and N released = (initial C (or N) content × dry initial mass - final C (or N) content × dry final mass) × aboveground biomass

2.3 Measurement of soil properties and assessment of ecosystem services

153 Soil pH was measured at a soil:water ratio of 1:2.5 (weight/weight). Air-dried soil and 25 ml of
154 deionized water were shaken together for 1 min and left to settle for 30 min, and the soil pH was
155 determined using an electrode. Soil organic carbon (SOC) was measured by titrimetry after oxidation
156 with a mixture of H_2SO_4 and $K_2Cr_2O_7$. Total N and P were determined using the Kjeldahl and
157 molybdenum blue colorimetric methods, respectively. All of these methods have been described by Lu
158 (2000).

159 Microbial biomass C (MBC) and microbial biomass N (MBN) were analysed using the
160 fumigation-extraction method. Ten grams of fresh soil was fumigated with chloroform in the dark for
161 24 h, and then the fumigated and nonfumigated soils were extracted with 0.5 M K_2SO_4 and shaken at
162 200 rpm for 0.5 h. Soil extracts were filtered through a 0.45-μm Millipore filter, and the C and N in the
163 extracts were determined using a multi C/N 3100 analyser (Analytik Jena AG). The C and N contents
164 in extracts of the nonfumigated soil were subtracted from C and N extracted from the fumigated soil to
165 give the C and N extracted from the soil microbial biomass. Values of 0.45 and 0.54 were used to
166 calibrate the contents of MBC and MBN, respectively (Vance et al., 1987; Wu et al., 1990).

167 The activities of cellulose and N-acetyl-β-glucosaminidase (NAG) were measured using
168 *p*-nitrophenyl-β-D-cellobioside and *p*-nitrophenyl-N-acetyl-β-D-glucosaminide as substrates,
169 respectively. Fresh soil (1.0 g) was mixed with 2.5 mL of 0.2 M acetate buffer (pH 5.0) and 2.5 mL of
170 0.02 M substrates and then shaken at 200 rpm and 37 °C for 1 h. The reaction was stopped by adding 1
171 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M Tris buffer (pH 12.0). The mixture was suspended with a vortex,
172 the supernatant was filtered, and the concentration of *p*-nitrophenol (PNP) was measured by
173 colorimetry at 400 nm. The same procedure was followed for the controls, with the exception that the
174 substrate was added after the incubation, and $CaCl_2$ and Tris buffer were added (Dick, 2011; Geisseler

and Horwath, 2009).

To estimate the greenhouse gas emission potential, we conducted a 60-day incubation experiment. Briefly, 20 g of fresh soil was placed in a 250-mL flask and then sealed with a gas-tight lid that had a rubber stopper in the middle. Gas samples (10 mL) were taken from the headspace of each flask at 1, 3, 7, 15, 30, and 60 days after sealing using a plastic syringe. The gas sample was immediately injected into a preevacuated 10-mL glass vial. Concentrations of methane (CH_4), N_2O and carbon dioxide (CO_2) were determined using a gas chromatograph (Agilent 7890) equipped with a flame ionization detector for CO_2 and CH_4 and a ^{63}Ni electron capture detector for N_2O. The gas standards were provided by the National Research Center for Certified Reference Materials, Beijing, China. The precision for greenhouse gas emission concentrations was ±0.5% based on repeated measurements of gas standards (Qiu et al., 2019). When the maize plants matured, all plants and grains were harvested from each plot, oven-dried at 60 °C for 48 h and weighed. Straw aboveground biomass and crop yield were converted into weight per hectare.

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We selected 15 soil properties to estimate cropland ecosystem services, i.e., the soil fertility index (SOC, total N, total P, MBC and MBN), greenhouse gas emission amount (mainly CO_2, N_2O and CH_4), straw decomposition and C and N released, soil extracellular enzymes (cellulase and N-acetyl-D-glucosaminidase), and maize biomass (aboveground biomass and crop yield). Generally, SOC, total N and total P are the major soil fertility factors and indicate the present nutrient status in croplands, which can be used to explain soil fertility conditions. Microbial biomass reflects ecosystem productivity. Greenhouse gas emissions are related to climate change, which can be regulated by fertilization regimes and soil microbial activities. Soil extracellular enzymes catalyse the decomposition of a range of organic polymers, resulting in C and N turnover. Maize biomass (such as

aboveground biomass and crop yield) reflects soil productivity. As a whole, all of these variables together contributed to the cropland function. To evaluate the function of the cropland ecosystem under different fertilization conditions, we calculated an integrative soil ecosystem multifunctionality index for further analysis. Notably, the opposite value was chosen for greenhouse gas emissions. Due to the lack of a specific definition of multifunctionality, we first calculated the *Z* scores of the 15 measured variables and obtained a multifunctionality value for each plot by averaging the *Z* scores of the 15 variables (Chen et al., 2019).

2.4 DNA extraction and quantification of general fungal ITS, bacterial 16S rRNA and genes encoding cellulose-degrading enzymes

Total DNA was extracted from 0.5 g freeze-dried soil by using a Fast DNA Spin Kit for Soil (MPbio, USA) according to the manufacturer's instructions and then dissolved in 50 μl of Tris-EDTA buffer. The quality of the DNA extraction was characterized by electrophoresis on 1% (wt/vol) agarose gels. The quantity and quality of DNA were checked using a Nanodrop spectrophotometer (Nanodrop, PeqLab, Germany). The extracted DNA samples were stored at −80 °C before molecular analysis.

Bacterial and fungal abundances were determined to reveal the changes in microbial community compositions. The abundances of bacteria and bacteria fungi were measured according to modified procedures (Fierer and Jackson., 2005). We selected the primers *338F/518R (338F: CCTACGGGAGGCAGCAG; 518R: ATTACCGCGGCTGCTGG)* and *NSI1/58A2R (NSI1: GTAGTCATATGCTTGTCT; 58A2R: CATTCCCCGTTACCCGTT)* for the qPCR assay. The thermal qPCR profiles for the bacteria and fungi were as follows: 95 °C 2 min for DNA denaturation, 35 cycles (95 °C 30 s, 60 °C 30 s, 72 °C 30 s, 80 °C 15 s) for DNA annealing, and 81 °C, 10 s for DNA extension; 95 °C 10 min for DNA denaturation, 40 cycles (95 °C 15 s, 52 °C 30 s, 72 °C 30 s, 79 °C 30 s) for

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DNA annealing, and 81 °C 10 s for DNA extension, respectively. The initial concentrations of the two plasmids used as the standards for the bacterial and fungal abundance analyses were 1.22×10^{10} and 9.05×10^{9}, respectively.

The fungal *cbhI* gene and bacterial *GH48* gene were selected as functional biomarkers of cellulolytic fungi and bacteria, respectively. The primers *GH48 F8*/*GH48 R5* (*GH48_F8*: 5 - GCCADGHTBGGCG ACTACCT - 3; *GH48_R5*: 5 - CGCCCCABGMSWWGTACCA - 3) and *cbhI F*/*cbhI R* (*cbhI F*: ACCAAYTGCTAYACIRGYAA; *cbhI R*: GCYTCCCAIATRTCCATC) were used for the qPCR assay. The abundance of bacterial *GH48* and fungal *cbhI* genes was quantified according to modified procedures (Zhang et al., 2017). The thermal profiles of qPCR for the target genes of *GH48* and *cbhI* were as follows: 95 °C, 5 min for DNA denaturation, 40 × (94 °C for 30 s, 60 °C for 45 s, and 72 °C for 90 s) for DNA annealing, and 84 °C, 10 s for DNA extension; and 94 °C, 4 min for DNA denaturation, 40 × (94 °C for 45 s, 50 °C for 30 s, and 72 °C for 60 s) for DNA annealing, and 81 °C, 10 s for DNA extension, respectively. The initial concentrations of the two plasmids as the standards for bacterial *GH48* and fungal *cbhI* gene abundance analysis corresponded to 1.85×10^{11} and 2.65×10^{10} copies g^{-1} dry soil, respectively. qPCR was performed in triplicate, and amplification efficiencies higher than 95% were obtained with r^2 values > 0.99.

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2.5 Bacterial 16S rRNA genes and fungal ITS amplification and sequencing

High-throughput sequencing was performed with the Illumina MiSeq sequencing platform (Illumina Inc.). Both the forward and reverse primers were tagged with an adapter and linker sequence, and 8-bp barcode oligonucleotides were added to distinguish the amplicons from different soil samples.

The primers *515F (5'-GTGCCAGCMGCCGCGGTAA-3')* and *907R (5'-CCGTCAATTCMTTTRAGTTT-3')* were chosen to amplify the 16S rRNA genes in the V4–V5

241 hypervariable region. PCR was conducted in a 50-μL reaction mixture containing 27 μL of ddH_2O, 2
242 μL (5 μM) of each forward/reverse primer, 2.5 μL (10 ng) of template DNA, 5 μL (2.5 mM) of
243 deoxynucleoside triphosphates, 10 μL of 5× Fastpfu buffer, 0.5 μL of bovine serum albumin, and 1 μL
244 of TransStart Fastpfu polymerase (TransGen, Beijing, China). The PCR conditions were 94 °C for 5
245 min; 30 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 30 s of extension; followed by 72 °C for
246 10 min (Caporaso et al., 2010).

247 The fungal ITS1 region was amplified using the primer pair *ITS1F*
248 *(CTTGGTCATTTAGAGGAAGTAA)/ITS2 (GCTGCGTTCTTCATCGATGC)* (Ghannoum et al., 2010).
249 The 50-μL reaction mixture of each reaction mix consisted of 1 μl (30 ng) of DNA, 4 μl (1 μM) of each
250 forward/reverse primer, 25 μl of PCR Master Mix, and 16 μl of ddH_2O. PCR amplification was
251 conducted at 98 °C for 3 min, followed by 30 cycles (98 °C for 45 s, 55 °C for 45 s, and 72 °C for 45 s),
252 with a final extension at 72 °C for 7 min (Ghannoum et al., 2010). All amplicons were cleaned and
253 pooled in equimolar concentrations in a single tube, after which they were subjected to library
254 preparation, cluster generation, and 250-bp paired-end sequencing on an Illumina MiSeq platform
255 (Illumina Inc., San Diego, CA, USA).

256 The raw sequence data were processed using the Qualitative Insights into Microbial Ecology
257 (QIIME) pipeline (Caporaso et al., 2010). Sequences that fully matched the barcodes were selected and
258 distributed into separate files for the bacterial 16S rRNA and fungal ITS genes. Poor-quality sequences
259 with lengths less than 200 bp (for fungal ITS) and 500 bp (for bacterial 16S) and quality scores less
260 than 20 were discarded, and the chimaeras were removed using the UCHIME algorithm (Edgar et al.,
261 2010). The remaining sequences were assigned to operational taxonomic units (OTUs) with a 97%
262 similarity threshold using UCLUST (Edgar, 2010). Alpha diversity and Bray–Curtis distances for

principal coordinate analysis of the soil microbial community were calculated after rarefying all samples to the same sequencing depth.

2.6 Statistical analysis

The soil ecosystem multifunctionality index, crop yields, microbial traits and other relevant soil variables among treatments were subjected to a chi-square test for independence of variance. Significant differences were determined by one-way analysis of variance (ANOVA) based on the post hoc Tukey test at the 5% level. Prior to ANOVA, the normality and homogeneity of variances were tested by the Kolmogorov–Smirnov test and Levene's test, respectively. If normality was not met, log or square-root transformation was implemented. One-way ANOVA was performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

Nonmetric multidimensional scaling (NMDS) analysis was used to describe and evaluate the microbial community composition. The NMDS was performed in the "Vegan" package of R (4.0.2). Analysis of similarities (ANOSIM) was used to examine the significant differences in microbial community structure under different fertilization.To describe the complex co-occurrence patterns in various organisms, we constructed co-occurrence networks. We focused on the abundant microbial phylotypes (with average relative abundance > 0.01% for bacteria and fungi) for network construction. Nodes with Pearson correlations greater than 0.70 and $p < 0.05$ were retained. Network visualization between microbial taxa and ecological clusters of microbial phylotypes were conducted and identified by Gephi software. To obtain the keystone species of each network, a *Zi-Pi* plot series was constructed to determine the role of each OTU. According to Deng et al. (2012), the plot includes (a) peripheral nodes ($Z \leq 0.25$, $P \leq 0.62$). (b) module hubs ($Z > 0.25$, $P \leq 0.62$), (c) connectors ($Z \leq 0.25$, $P > 0.62$) and (d) network hubs ($Z > 0.25$, $P > 0.62$). From an ecological perspective, OTUs in module hubs,

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connectors and network hubs may be regarded as the microbial keystone taxa of the network systems (Deng et al., 2015).

The first heatmap was constructed to reveal the associations between soil ecosystem servises with microbial module comminuties. And another heatmap was constructed to reveal the associations between microbial traits and fertilizers, soil properties, greenhouse emissions and ecosystem multifunctionality. The random forest algorithm was performed in the R package (4.0.2) "RandomForest" to estimate the importance predictors of soil properties and microbial traits on ecosystem multifunctionality.

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3. Results

3.1 Cropland ecosystem services

Data collection after a continuous 4-year in situ field experiment under different N input levels revealed changes in cropland ecosystem services (Fig. 1). In terms of soil fertility, compared with the N-limitation treatments (PK and 0.5N+PK), the SOC and total P contents were increased significantly by the N+PK and 0.75N+PK treatments (Fig. 1a, c) ($P < 0.05$), while there were no significant changes in the total N content (Fig. 1b). After straw decomposition (Fig. 1d), the amounts of straw C (Fig. 1e) and N (Fig. 1f) released showed different responses to varying N fertilizer input levels. Generally, N-rich treatments (N+PK and 0.75N+PK) significantly increased the straw decomposition rate and achieved higher amounts of straw C and N release than the N-limitation treatments ($P < 0.05$). However, there was no significant difference between N+PK and 0.75N+PK. Microbial biomass C and N content, as well as associated enzyme activity were changed after different N fertilizer application rates (Fig. 1g, h, i and j). The MBC (Fig. 1g) and MBN (Fig. 1h) contents were significantly higher in the N-rich treatments than in the other treatments. However, the highest cellulase activity was observed

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in the 0.75N+PK treatment, which was significantly higher than that in the other treatments (Fig. 1i) ($P < 0.05$), and the N-acetyl-D-glucosaminidase activity decreased with the reduction in N application (Fig. 1j).

For greenhouse gas emissions, with the decrease in N fertilizer application levels, CO_2 and N_2O emissions gradually decreased (Fig. 1k, m). No significant difference was observed in CH_4 emissions under the different fertilization treatments (Fig. 1l). In addition, the N fertilizer levels also had a strong influence on maize yields and aboveground biomass (Fig. 1n, o). As expected, the 0.75N+PK treatment achieved the highest multifunctionality index (0.61), followed by N+PK (0.32), 0.5N+PK (-0.34) and PK (-0.59) (Fig. 1p).

However, although the 0.75N+PK treatment increased the straw N release amount and may meet the requirements for plant growth, the total N input was still dominated by inorganic N input (Fig. S1). Therefore, the N released from the straw cannot offset the deficiency of N fertilizer. Additionally, contrasting N fertilizer input levels significantly changed the stoichiometry of C, N and P (Fig. S2). Notably, the 0.75N+PK treatment significantly increased the C:N ratio compared with the 0.5N+PK and PK treatments ($P < 0.05$). The lowest C:N ratio was shown for the 0.5N+PK treatment (Fig. S2a). The N:P and C:P ratios showed no significant difference regardless of nutrient excess or limitation (Fig. S2b and c).

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删除[DY]: Our results indicated that the N+PK and 0.75N+PK treatments resulted in higher maize yields and aboveground biomass than the other treatments ($P < 0.05$), suggesting that a 25% N fertilizer reduction could be satisfactory for maize growth.

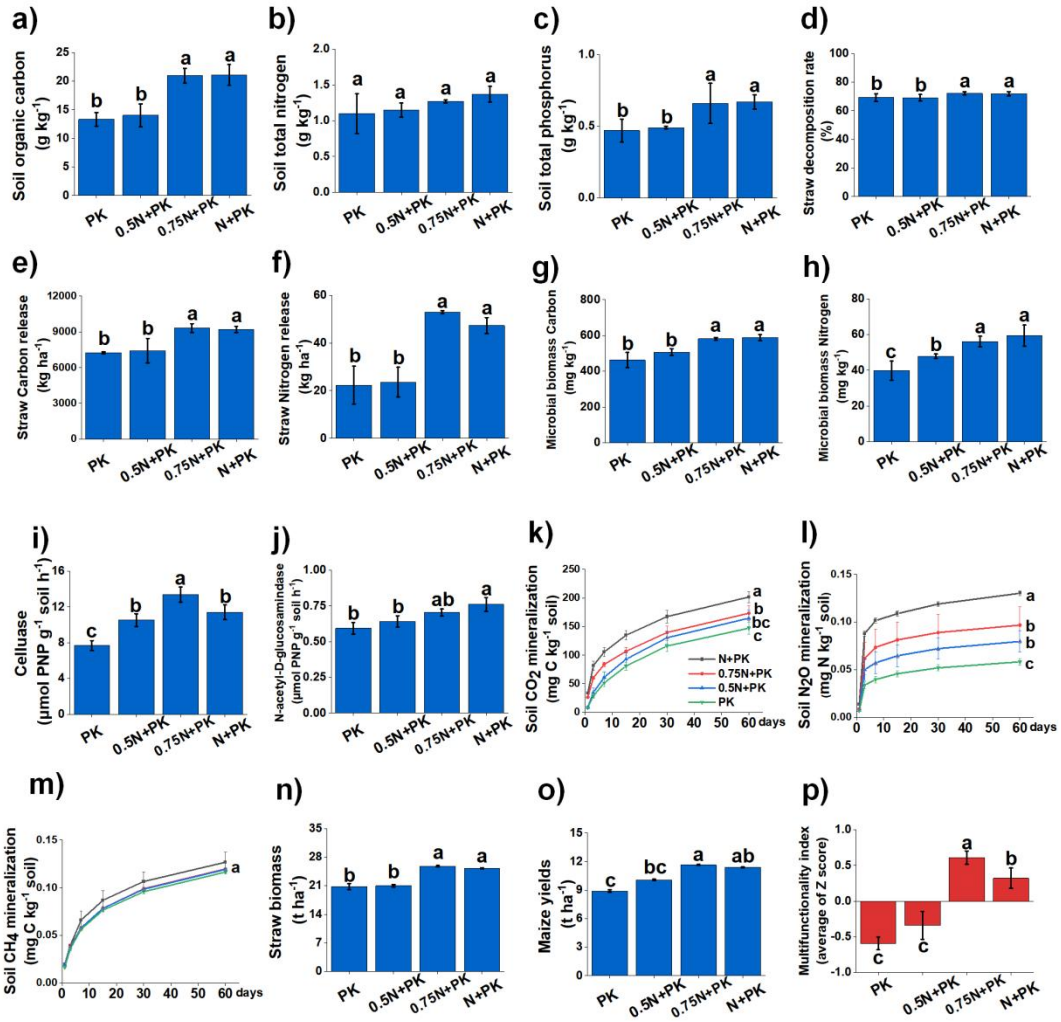


Fig. 1 The 15 cropland variables and multifunctionality index under different N input levels after straw return. Abbreviations: N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.

3.2 Abundances of bacteria, fungi and genes encoding cellulose-degrading enzymes

N fertilizer input levels had marked impacts on the abundances of fungi and bacteria (Table S3). The highest fungal abundance was observed in the 0.75N+PK treatment, which was significantly higher than that in the other treatments ($P < 0.05$). The N+PK treatment significantly increased bacterial abundance compared with the PK treatment ($P < 0.05$), while there were no obvious

differences among the N+PK, 0.75N+PK and PK treatments. The ratios of fungi to bacteria also showed contrasting responses to N fertilization (Table. S3). The 0.75N+PK treatment significantly increased the ratio of fungi to bacteria compared with the other treatments ($P < 0.05$), and the lowest ratio of fungi to bacteria was found in the PK treatment.

Table 1 The abundances of genes encoding ellulose-degrading enzymes across different N fertilizer level treatments after straw return

Treatment	*cbhI* gene abundance ($\times 10^6$ copies g^{-1} soil)	*GH48* gene abundance ($\times 10^7$copies g^{-1} soil)	*cbhI*: *GH48* ratio
N+PK	**4.75±0.16 a**	**1.68±0.01 a**	**0.28±0.01 a**
0.75N+PK	**4.95±0.19 a**	**1.60±0.04 a**	**0.31±0.02 a**
0.5N+PK	**4.01±0.12 b**	**1.54±0.08 a**	**0.26±0.03 b**
PK	**3.76±0.13 b**	**1.40±0.06 b**	**0.27±0.02 b**

The results show means ± standard deviations (n = 3). Different lowercase letters after values indicate significant differences between each treatment, $P < 0.05$. N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction ; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.

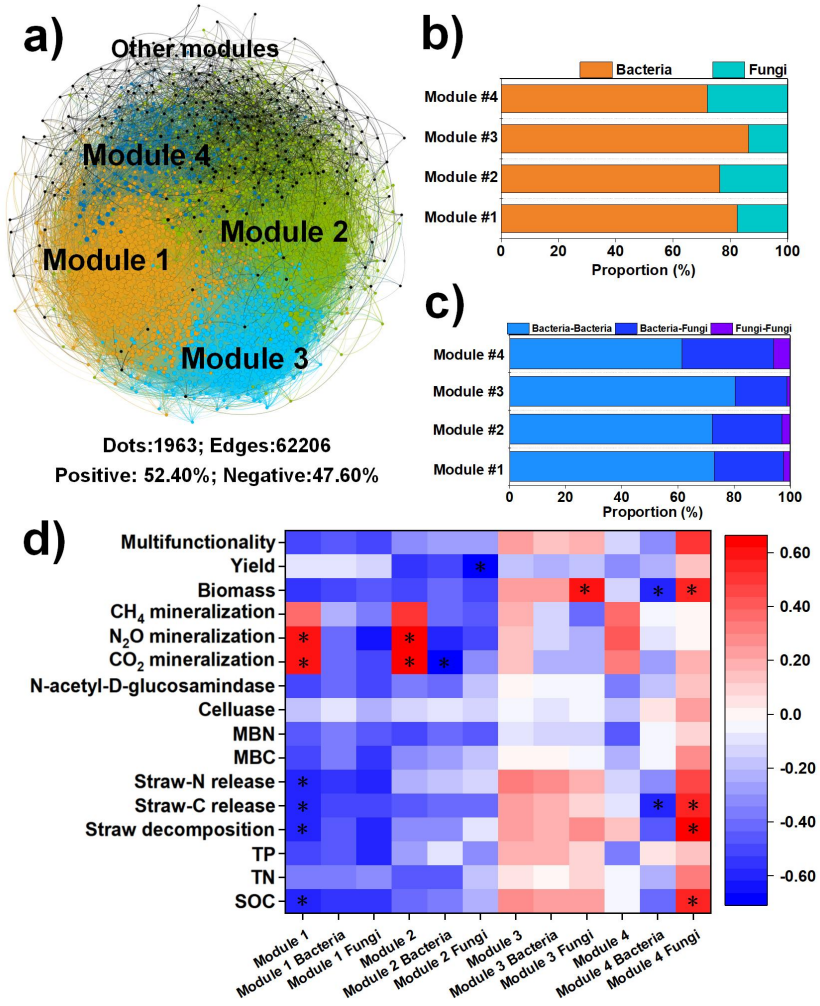
N fertilizer input levels led to changes in the expression levels of genes encoding cellulose-degrading enzymes (Table 1). The N-rich treatments achieved higher fungal *cbhI* and bacterial *GH48* gene abundance than the N-limitation treatments. In contrast, the highest *cbhI* gene abundance was shown in the 0.75N+PK treatment, while the highest *GH48* gene abundance was shown in the N+PK treatment. Compared with the PK treatment, the ratio of the fungal *cbhI* gene to the

352 bacterial *GH48* gene increased significantly under the 0.75N+PK treatment ($P < 0.05$).

353 **3.3 Co-occurrence network analysis of the microbial community**

354 Regarding fungal alpha diversities, there were no significant differences in the Chao1 index across
355 treatments. The N+PK treatment significantly increased fungal richness compared with the PK
356 treatment ($P < 0.05$) (Table S4). In addition, the PK treatment resulted in lower bacterial richness than
357 the other treatments ($P < 0.05$). No significant difference was observed in the bacterial Chao1 index
358 across treatments (Table S4). NMDS plots showed that diverse N input levels significantly changed the
359 fungal (Fig. S3a) and bacterial communities (Fig. S3b) ($P < 0.05$).

360 We further conducted network analysis to identify co-occurrence patterns between specific
361 microbial taxa (Fig. 2). The cooccurrence network was aggregated into smaller coherent modules that
362 were examined to determine important module-trait relationships. The present network comprised 1963
363 nodes (composed of 1520 bacterial taxa and 443 fungal taxa) and 62206 edges with 52.49% positive
364 associations (Fig. 2a). The results showed four dominant ecological modules (1-4) that strongly
365 co-occurred within the multitrophic network, which contributed 86.10% of the whole network. Among
366 the four modules, bacteria accounted for the highest proportion in each module, contributing more than
367 70% of the total (Fig. 2b). The percentage of edges linking bacteria to bacteria (B-B) was higher than
368 that linking fungi to fungi (F-F) and bacteria to fungi (B-F). The highest proportion of B-B (80.32%)
369 was found in Module 3, while the highest proportion of B-F (32.66%) and F-F (6.00%) was found in
370 Module 4 (Fig. 2c).
371



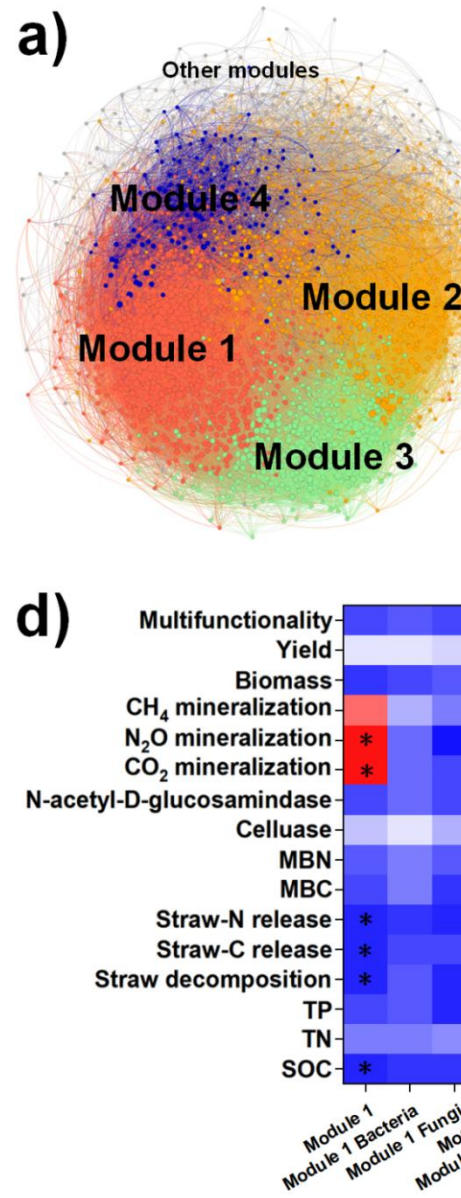


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Fig. 2 The relationships of microbial module communities with soil ecosystem services and dis-services. Multitrophic network including multiple ecological modules. The colours of the nodes represent different ecological modules (a). OTU number proportions of bacteria and fungi (b). The proportions of the edges linking bacteria to bacteria (B-B), bacteria to fungi (B-F) and fungi to fungi (F-F) in the major ecological modules (c). Links between the specific module communities with soil ecosystem services and dis-services (d). * indicates significance at $P < 0.05$. Abbreviations: SOC, soil organic carbon; C: N, the ratio of the SOC content to the total N content; N: P, the ratio of the total N content to the total P content.

Individual nodes represented different roles in the microbial network based on the intramodule connectivity *Zi* and the intermodule connectivity *Pi*. *ZP* plots were constructed to identify the topological roles of each node in the network (Fig. 3a). As shown in Fig. 3b, 113 microbial taxa (81 bacterial species and 32 fungal species) were regarded as connectors, and 43 microbial taxa (39 bacterial species and 4 fungal species) were regarded as module hubs. Specifically, module 2 (54) contained the most keystone taxa, followed by module 1 (38) and module 3 (32).

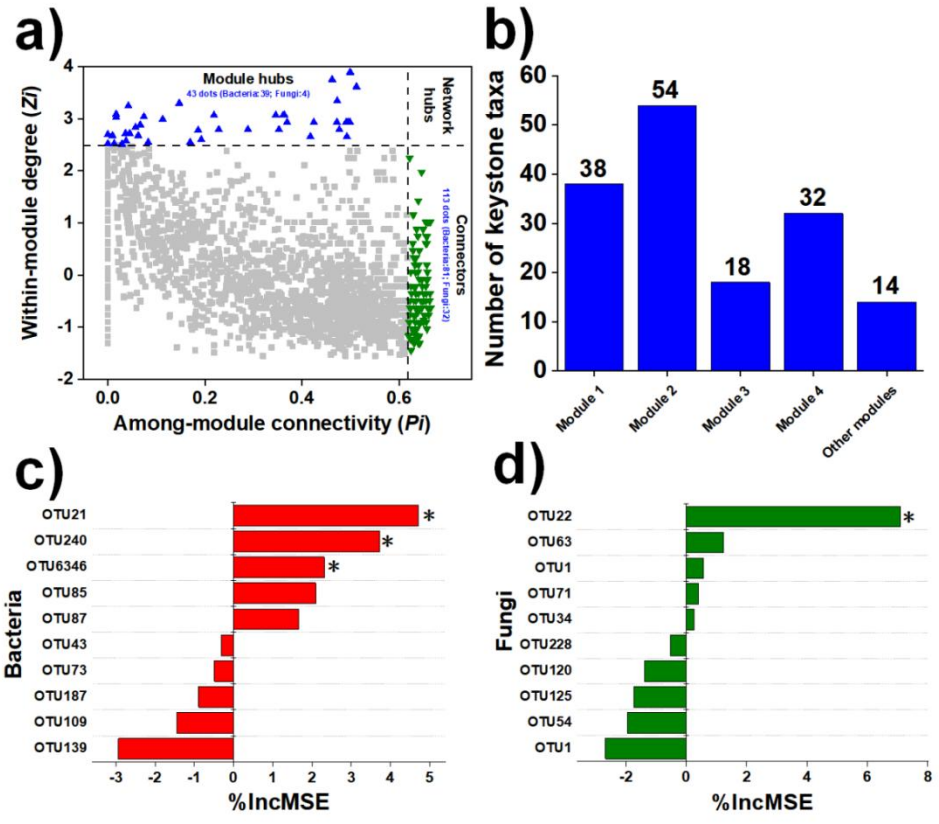

Fig. 3 The topological roles of microbial taxa and their effect on the soil multifunctionality index. The topological role of each OTU was determined according to the scatter plot of within-module connectivity (*Z*) and among-module connectivity (*P*) (a). The distribution of keystone taxa in each ecological module (b). Contribution of bacterial (c) and fungal OTUs (d) to the soil multifunctionality index. *, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001, respectively.

3.4 Linkage between microbial traits and soil ecosystem multifunctionality

The heatmap showed the close correlations between fertilizers (N input and straw return) with soil stoichiometry and microbial traits (Fig. 4). Overall, the N input level, straw biomass and C:N ratio upregulated the abundance of genes encoding cellulose-degrading enzymes. In addition, N input was positively correlated with bacterial abundance, while a significant correlation was observed between straw biomass and the N input level. The random forest model was also used to identify abiotic and biotic attributes correlated with soil ecosystem multifunctionality (Fig. 5). The model explained 83.89% of the variance in ecosystem multifunctionality. The results indicated that the N input level, straw biomass and soil C:N ratio were the most prominent abiotic factors affecting the ecosystem multifunctionality index, while some biotic factors, such as the abundance of genes encoding cellulose-degrading enzymes, significantly affected the ecosystem multifunctionality index.

Moreover, to clarify the potential main specific drivers of soil ecosystem services, correlations between the microbial physiological traits and soil properties were determined to illuminate the role of the microbial community in soil ecosystem multifunctionality (Fig. 2d). The results indicated that the particular microbial module community was significantly correlated with soil ecosystem services. The communities of modules 1 and 2 and the fungal community in module 4 showed potential in soil ecosystem services (Fig. 2d). Specifically, significant correlations were observed between the SOC content, straw decomposition, straw C/N release, CO_2/N_2O mineralization and the module 1 community; the module 2 community was positively correlated with greenhouse gas emissions (except for CH_4); and the fungal community in module 4 was positively correlated with the SOC content, straw decomposition, straw C/N release and straw biomass. Furthermore, the bacterial and fungal communities belonging to module 2 and the fungal community belonging to module 3 were

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significantly correlated with CO_2 emission, maize yield and straw biomass.

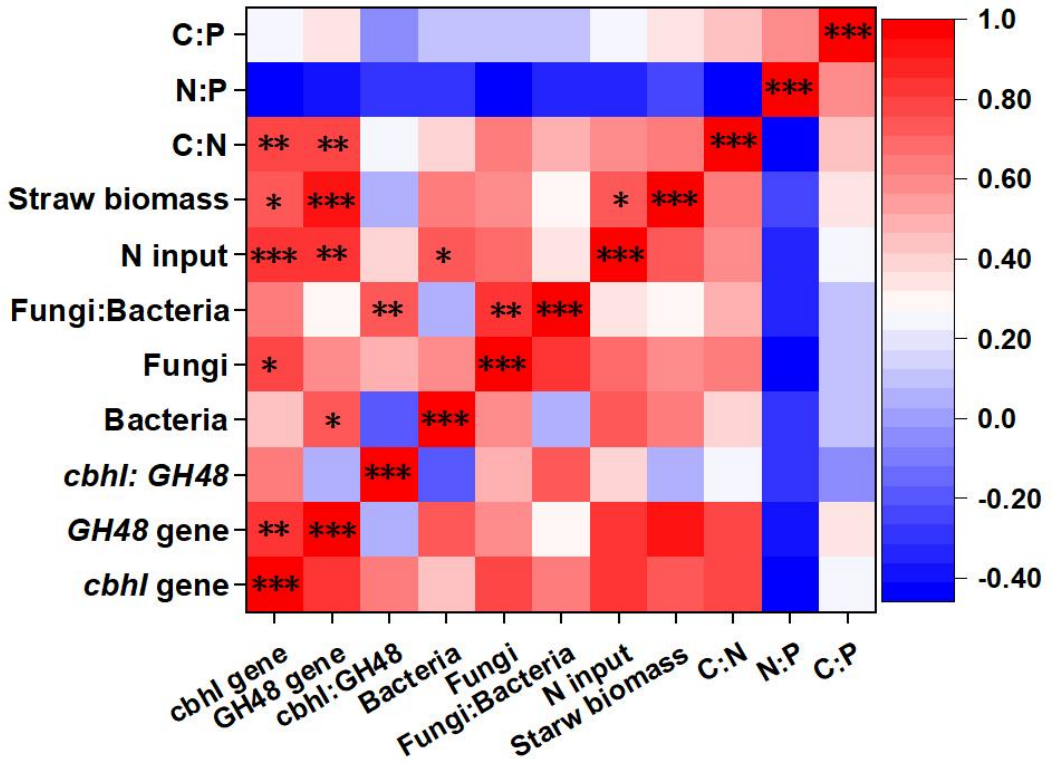

Fig. 4 Heatmap revealing the correlation coefficients between microbial traits with fertilization and soil stoichiometry. *, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001, respectively. Abbreviations: C: N, the ratio of the SOC content to the total N content; N: P, the ratio of the total N content to the total P content.

At the scale of microbial species, we selected the 20 keystone taxa (10 bacterial and 10 fungal taxa) with the highest relative abundance for further analysis. The random forest models indicated that the specific keystone taxa strongly influenced soil ecosystem multifunctionality (Fig. 3c and d). Bacterial OTU21 (in module 1), OTU240 (in module 2) and OTU6346 (in module 3) were highlighted as essential predictors of soil ecosystem multifunctionality, and fungal OTU22 (module 3) was also found to be an important variable for predicting its changes. Subsequently, the relative abundances of selected keystone taxa were different across different N fertilizer level treatments after straw return

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(Table S5). The relative abundances of fungal OTU22 and bacterial OTU21 were higher in the N-rich treatments than in the N-limitation treatments. Moreover, compared with the N+PK treatment, the 0.75N+PK treatment increased the relative abundances of fungal OTU22 by 38.20% and bacterial OTU21 by 40.63%.

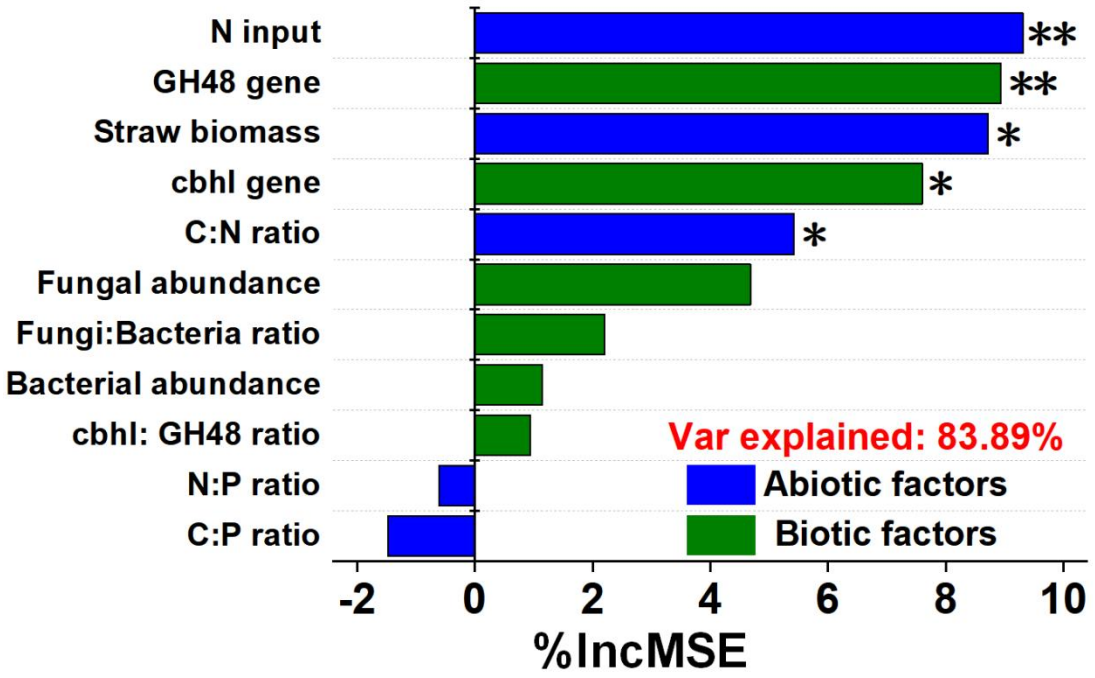

Fig. 5 Contribution of abiotic and biotic variables to the soil multifunctionality index. *, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001, respectively. Abbreviations: C: N, the ratio of the SOC content to the total N content; N: P, the ratio of the total N content to the total P content.

4. Discussion

4.1 Effect of N fertilizer reduction on cropland ecosystem services after straw return

Soil fertility, straw decomposition, C and N release amounts, and crop productivity were mostly higher under 0.75N+PK and N+PK than other treatments, implying that better soil multifunctionality was achieved. Moreover, N+PK increased greenhouse gas emissions (Fig. 1). Higher microbial

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删除[DY]: Our results indicated that the soil fertility index (SOC, total N and P contents) increased under N-rich treatments as a result of high net primary production, in accordance with previous reports (Liu et al., 2010; Williams et al., 2013).

biomass C and N, as well as relevant enzyme activities, were also observed under N-rich treatments, indicating the strong positive impact of abundant N fertilizer application (Fig. 1g, h, i, j). It was reported that straw return with N fertilizer application can stimulate microbial activity and promote biomass accumulation (Treseder, 2008). The substantially increased straw decomposition and straw C and N release under N-rich treatments may be primarily attributed to the activation of microbial activity (Fig. 1d, e, f), which is consistent with previous research (Ramirez et al., 2012). Our results indicated that 0.75N+PK maintained soil fertility index net primary production compared to N+PK. This study demonstrates that 0.75N+PK has similar effects on soil ecosystem services as N+PK. Therefore, it can be concluded that 0.75N+PK is a more efficient and effective option for improving soil ecosystem services. Moreover, 0.75N+PK may enhances N fertilizer use efficiency and stimulates microbial functioning by modulating the stoichiometry of C,N and P in the soil, which promotes soil fertility and crop yield (Liu et al., 2010). Reducing the amount of N fertilizer by more than 50% led to insufficient N input to meet the needs of both crops and microbes, resulting in a decline in soil health (Williams et al., 2013). Recent studies have also proven that rational N input can stimulate microbial ex vivo production of extracellular enzymes to accelerate straw decomposition and nutrient transformation (Chen et al., 2016). Moreover, it is well known that fungi have high nutrient utilization efficiency; thus, more straw-derived C and N would be stored in soil under N-rich treatments than under N-limited treatments (Hou et al., 2020). Rational N availability is also the premise of straw decomposition and SOM formation due to the microbial “stoichiometry decomposition” theory, while the “N-mining” theory in N-limitation treatments reveals that oligotrophic species (such as *K*-strategists) degrade native SOM because of the lack of N fertilizer inputs (Chen et al., 2014). In this study, a 25% reduction in N fertilizer application may be the threshold value for the “N-mining” and the “stoichiometry

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decomposition". Finally, the increases in SOC, total N, and P contents and straw C and N release, as well as microbial biomass and function, are commonly attributed to high aboveground biomass and maize yields (Fig. 1n, o), which are favourable from the viewpoint of ecosystem services.

However, the overuse of N inputs also causes more greenhouse gas emissions (Tang et al., 2019). In the present study, greenhouse gas emissions were quantified to evaluate the ecosystem dis-services under different N fertilizer input levels, which means the larger the value, the lower the soil ecosystem multifunctionality (Fig. 1k, l, m). Straw return with N fertilizer addition might be the crucial driver of CO_2 and N_2O emissions from agroecosystems and has been widely studied in previous literature (Gregorich et al., 2005). CO_2 and N_2O emissions increased significantly compared with those under the PK treatment, likely by stimulating the activity of copiotrophs when sufficient C and N substrates were provided. For example, Dieleman et al. (2010) implied that an increase in CO_2 and N_2O as the amount of N input increases through meta-analysis and field experiments, respectively. Qiu et al. (2019) indicated that the emission of CO_2 enhanced root and mycorrhizal N uptake and increased N_2O emissions, which was related to the changes in the soil denitrifier community composition in favour of N_2O-producing taxa (nirK- or nirS-type). In addition, there was no difference in CH_4 emissions among treatments, although contradictory results have been widely reported in previous literature (Tang et al., 2019). Mapanda et al. (2011) and Liu et al. (2012) indicated that the emission of CH_4 depended highly on the soil water content in maize crops, which is in line with our results. To summarize, previous researches clearly demonstrated a positive correlation between CO_2 and N_2O emissions with N input. So, this study unequivocally showed that N+PK emits more greenhouse gases than 0.75N+PK.

In summary, compared with the N+PK treatment, the 0.75N+PK treatment supported multiple ecosystem services, including promoting soil fertility, straw nutrient release and microbial activity and

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alleviating greenhouse gas emissions (Fig. 1p). Therefore, a reduction of 25% in chemical N fertilizer input with straw return may be the appropriate regime to promote ecosystem services in meadow soils on the Northeast China Plain.

4.2 Responses of the microbial abundance and function to straw return with N fertilizer reduction

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Fungal and bacterial abundances, as well as the ratio of fungi to bacteria, were sensitive to the changes in the N fertilizer input levels (Table S3 and Fig. 2). Straw addition with N fertilizer input supplied enough C and N for microbial metabolism, thus promoting microbial proliferation (Chen et al., 2016). Generally, bacterial abundance decreased with reduced N fertilizer input. This is mainly because bacteria are more sensitive to N availability than fungi, which is in line with a previous study (Ramirez et al., 2020). It is worth noting that a 25% reduction of N fertilizer significantly increased fungal abundance compared with regular N inputs. This result might be attributed to the negative effect of excess N fertilizer (Wan et al., 2015). Moreover, Ning et al. (2020) demonstrated that the C:N ratio was the pivotal factor in fungal community compositions after performing 7 long-term field experiments under different fertilization conditions across China and reported a significant positive correlation between them. Gao et al. (2015) indicated that the rational ratio of C and N input was 20:1, which may meet the demand of maize growth and microbial proliferation. It is well known that fungi have a strong C utilization efficiency compared to bacteria (Duan et al., 2021). Therefore up-regulation of fungal abundance and lowering the ratio of bacteria to fungi are crucial for straw degradation and SOC accumulation. Previous studies have shown that the C: N ratio of fungi is greater than 20, however, the C: N ratio of bacteria is less than 10. Excessive N fertilizer input may reduce the soil C: N ratio, while little N fertilizer input can not meet the growth requirements of crops and microorganisms (Ning et al.,

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2020). Therefore, appropriate enhancement of soil C: N ratio can increase the ratio of fungi to bacteria, stimulate fungal function, and promote straw degradation and SOC accumulation. Therefore, the 0.75N+PK treatment with a higher C:N ratio (16.47) may facilitate the proliferation of microorganisms and promote an increase in microbial abundance.

Subsequently, our results showed that N-rich treatments resulted in higher microbial cellulose-degrading gene abundances than the PK treatment (Table 1), which demonstrated the irreplaceable role of N inputs in straw degradation (Zhang et al., 2017). Additionally, compared with bacterial *GH48* gene abundance, the increase in fungal *cbhI* gene abundance required adequate N fertilizer inputs and was regulated by the soil C:N ratio, which suggests that rational N fertilizer inputs could promote fungal function for further degradation of recalcitrant straw components (Hou et al., 2020). Therefore, the ratio of *cbhI* gene abundance to *GH48* gene abundance was higher under 0.75N+PK than under the N-limitation treatments since the increased expression of a fungal cellulose-degrading gene implies more straw C and N release.

Our results indicated that 75%-100% N fertilizer could upregulate fungal and *cbhI* gene abundances, which may lead to straw decomposition and SOC accumulation. It is therefore necessary to further explore the potential associations between microbial traits and ecosystem services under diverse N fertilizer input levels.

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4.3 Linkages of cropland ecosystem services with microbial traits

To clarify the effect of abiotic and biotic factors on soil ecosystem services, we then quantified the contributions of abiotic and biotic attributes to the ecosystem multifunctionality index across N input treatments (Fig. 4 and 5). Biotic factors, such as *cbhI* and *GH48* gene abundances, as well as abiotic factors, including the C:N ratio, straw biomass and N input level, are also pivotal regulators of

ecosystem multifunctionality (Fig. 5). In general, promoting the rapid degradation of straw is an important way to convert straw-C into SOM, thus improving soil fertility, aboveground biomass and crop yield. In addition, fungi have a higher C utilization efficiency than bacteria; thus, a high fungal *cbhI* gene abundance may achieve better soil multifunctionality (Hou et al., 2020). For abiotic factors, the soil C:N ratio, straw biomass and N fertilizer input are always regarded as the main indicators of soil fertility and health, likely due to providing various nutrient accessibilities and influencing the microbial community composition (Ning et al., 2020).

Numerous studies have shown that core microbiota play a vital role in maintaining the stability of soil microbial function and the complexity of microbial networks and then promoting soil nutrient cycling ecosystem services (Ghannoum et al., 2015), and keystone species may show great explanatory power in terms of specific network (or module) structure and function (Chen et al., 2019b). In the present study, *Terrimonas* (bacterial species in module 1) and *Lasiosphaeriaceae* (fungal species in module 3) were detected as the keystone taxa in influencing soil multifunctionality of the cooccurrence network (Table S5). A previous study demonstrated that straw addition significantly increased the relative abundance of *Lasiosphaeriaceae*, which implied straw decomposition ability (Song et al., 2020). Afterwards, *Lasiosphaeriaceae* was proven to promote straw-derived C and N accumulation by secreting multiple extracellular enzymes (Guo et al., 2022). Meanwhile, Sun et al. (2023) revealed that *Lasiosphaeriaceae* abundance was regulated by the soil C:N ratio, especially changes in mineral N. Therefore, *Lasiosphaeriaceae* can effectively promote straw degradation and straw C and N release while driving the function and community of module 1, which is consistent with our results (Fig. 2d). However, relatively few studies have focused on the function of *Terrimonas*, so this study focused on *Chitinophagaceae*. As reported in the previous literature, straw return was the main method to increase

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553 *Chitinophagaceae* abundance (Li et al., 2021). Furthermore, *Chitinophagaceae* was indicated to have a
554 strong ability to accumulate soil C and N and degrade cellulose (Zhong et al., 2022), facilitating
555 production improvement by regulating the module 3 community and function, which is in line with our
556 results (Fig. 2d).

557 Overall, straw return with sufficient N fertilizer application can increase the C:N ratio and
558 stimulate microbial traits, which ultimately achieve soil ecosystem multifunctionality (Fig. 6). Straw
559 return without enough N supply cannot support ecosystem services due to the decomposition of native
560 SOM and the out-of-balance microbial community composition, according to the "N-mining" theory
561 (Chen et al., 2014); straw return with sufficient N application (N+PK and 0.75N+PK) can promote soil
562 fertility, straw release, microbial activity and crop productivity, which can be explained by the
563 "stoichiometry decomposition" theory (Chen et al., 2014). Meanwhile, N+PK also caused more serious
564 ecosystem dis-services, such as greenhouse gas emissions, than the 0.75N+PK treatment. Moreover,
565 compared with the N+PK treatment, the 0.75N+PK treatment increased the soil C:N ratio and
566 stimulated microbial module 1 and 3 communities function, *cbhI* gene abundance, and keystone taxa
567 abundances, which were significantly positively correlated with soil ecosystem multifunctionality.
568 While *Lasiosphaeriaceae*-driven module 1 and *Terrimonas*-driven module 3 communities may be
569 involved in maintaining soil ecosystem multifunctionality. Our study provides evidence that a 25%
570 reduction of chemical N fertilizer after straw return was the optimal agronomic measure for ecosystem
571 services in meadow soil on the Northeast China Plain.

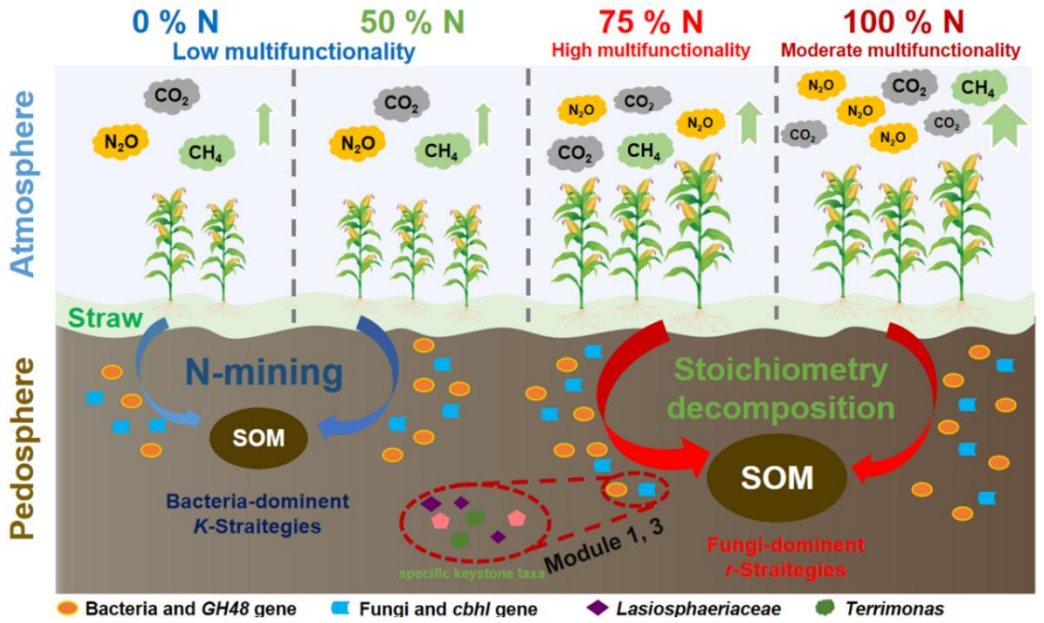

Fig. 6 A graphical sketch of the changes in ecosystem services and potential microbial mechanisms in response to different chemical N fertilizer application rates after straw return. N, nitrogen; SOM, soil organic matter

5. Conclusion

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Straw return combined with different chemical N fertilizer application rates significantly changed ecosystem services and dis-services. Collectively, our work indicates that compared with the N+PK treatment, straw return with a 25% reduction in chemical N fertilizer has the potential to improve ecosystem services by maintaining soil fertility, productivity, microbial biomass and function, promoting straw decomposition and C and N release and alleviating greenhouse gas emissions. The 0.75N+PK treatment achieved higher soil ecosystem multifunctionality than all other treatments. In addition, the N input level, straw biomass and soil C:N ratio can upregulate the abundances of the *cbhI* and *GH48* genes, which may together achieve soil ecosystem multifunctionality.

Furthermore, the changes in multiple soil ecosystem services were strongly associated with microbial module communities and keystone taxa. The relationships between ecosystem services and microbial traits were examined here to confirm that the *Lasiosphaeriaceae* driving the function and

structure of the module 1 community leads to the promotion of straw degradation and straw C and N release, while *Terrimonas* driving the function and structure of the module 3 community probably contributes to production improvement under 0.75N+PK treatment. Therefore, a 25% reduction in chemical N fertilizer with straw return might be a win–win strategy that not only produces considerable ecological benefits for the pedosphere and atmosphere but also reduces fertilizer expenditures in meadow soil on the Northeast China Plain.

Author contributions

YD, LFW, and XHM designed the experiment; YD, HMC, ZN, WLZ, and YMW performed the measurements; YD, YMC, MXZ, and JYL analyzed the data; YD and MHC wrote the manuscript draft; YML, JYL and LFW reviewed and edited the manuscript.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure captions

Fig. 1 The 15 cropland variables and multifunctionality index under different N input levels after straw return. Abbreviations: N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.

Fig. 2 The relationships of microbial module communities with soil ecosystem services and dis-services. Multitrophic network including multiple ecological modules. The colours of the nodes represent different ecological modules (a). OTU number proportions of bacteria and fungi (b). The proportions of the edges linking bacteria to bacteria (B-B), bacteria to fungi (B-F) and fungi to fungi (F-F) in the major ecological modules (c). Links between the specific module communities with soil ecosystem services and dis-services (d). * indicates significance at $P < 0.05$. Abbreviations: SOC, soil organic carbon; C: N, the ratio of the SOC content to the total N content; N: P, the ratio of the total N content to the total P content.

Fig. 3 The topological roles of microbial taxa and their effect on the soil multifunctionality index. The topological role of each OTU was determined according to the scatter plot of within-module connectivity (Z) and among-module connectivity (P) (a). The distribution of keystone taxa in each ecological module (b). Contribution of bacterial (c) and fungal OTUs (d) to the soil multifunctionality index. *, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001, respectively.

Fig. 4 Heatmap revealing the correlation coefficients between microbial traits with fertilization and soil stoichiometry. *, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001,

respectively. Abbreviations: C: N, the ratio of the SOC content to the total N content; N: P, the ratio of the total N content to the total P content.

Fig. 5 Contribution of abiotic and biotic variables to the soil multifunctionality index. *, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001, respectively. Abbreviations: C: N, the ratio of the SOC content to the total N content; N: P, the ratio of the total N content to the total P content.

Fig. 6 A graphical sketch of the changes in ecosystem services and potential microbial mechanisms in response to different chemical N fertilizer application rates after straw return. N, nitrogen; SOM, soil organic matter

Table 1 The abundances of genes encoding ellulose-degrading enzymes across different N fertilizer level treatments after straw return

Treatment	*cbhI* gene abundance ($\times 10^6$ copies g^{-1} soil)	*GH48* gene abundance ($\times 10^7$copies g^{-1} soil)	*cbhI*: *GH48* ratio
N+PK	4.75±0.16 a	1.68±0.01 a	0.28±0.01 a
0.75N+PK	4.95±0.19 a	1.60±0.04 a	0.31±0.02 a
0.5N+PK	4.01±0.12 b	1.54±0.08 a	0.26±0.03 b
PK	3.76±0.13 b	1.40±0.06 b	0.27±0.02 b

The results show means ± standard deviations (n = 3). Different lowercase letters after values indicate significant differences between each treatment, $P < 0.05$. N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction ; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.

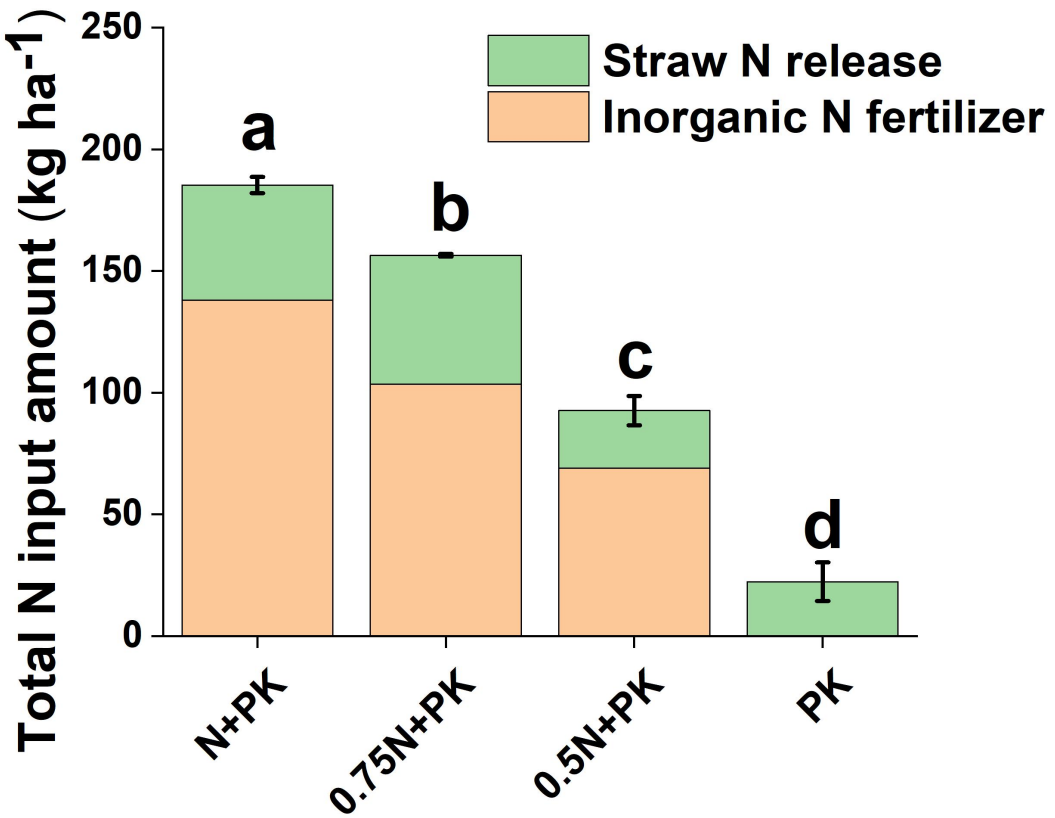

Fig. S1 The total N fertilizer input amount under different treatments. Different letters indicate significant differences at the level of $P < 0.05$. N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.

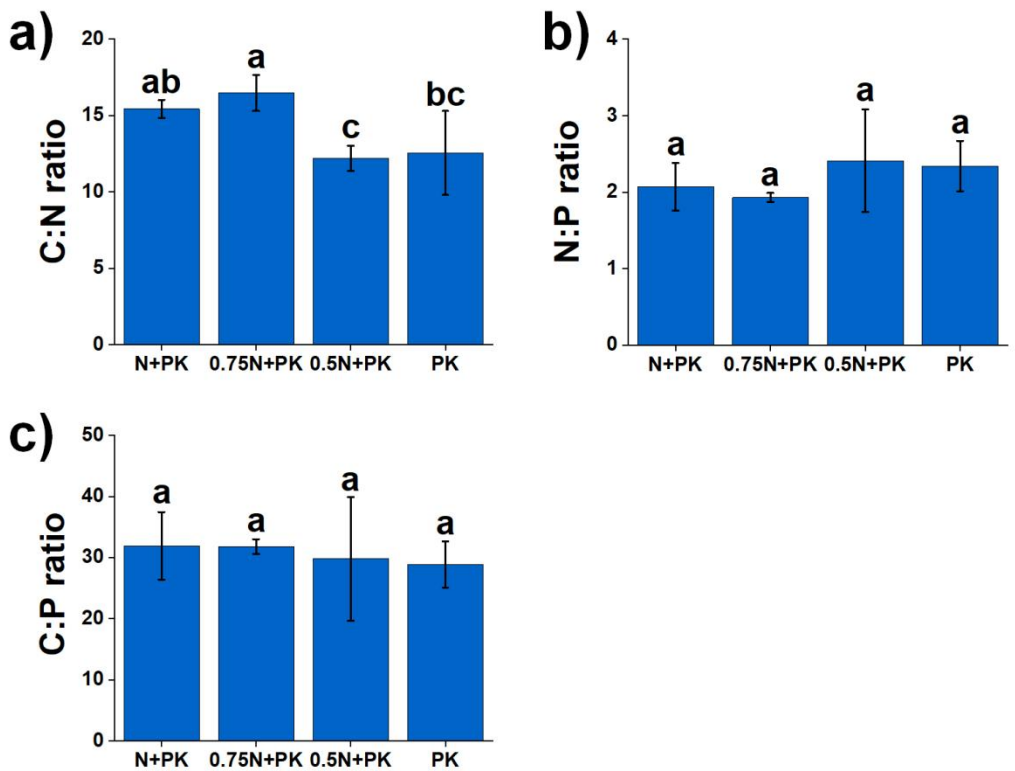

Fig. S2 The soil C:N ratio (a), N:P ratio (b) and C:P ratio (c) under different treatments. Different letters indicate significant differences at the level of $p < 0.05$. N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.

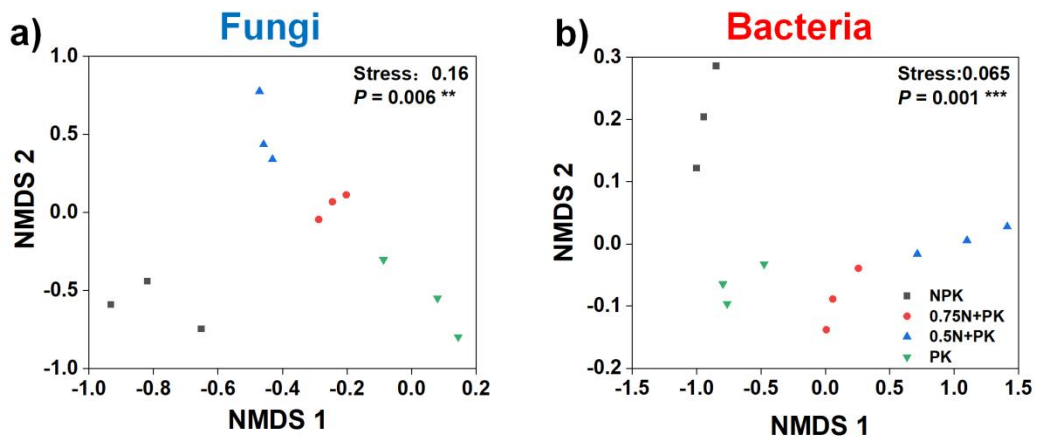

Fig. S3 Non-metric multidimensional scaling ordination showing the fungi (a) and bacteria (b) under different N input levels; significant differences in sample clustering are measured by ANOSIM. N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.

Table S1 The yields and soil chemical properties under different treatments of bulk soil during the experimental process

Year	Treatment	Yield (t ha^{-1})	pH	SOC (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})
2019	N+PK	11.17±0.73 a	7.29±0.14 a	17.60±2.10 a	0.93±0.02 a	0.65±0.08 a
	0.75N+PK	10.91±0.29 a	7.31±0.20 a	15.87±3.12 ab	0.94±0.08 a	0.61±0.12 a
	0.5N+PK	9.81±0.32 b	7.39±0.18 a	14.01±1.42 b	0.89±0.10 a	0.64±0.03 a
	PK	9.93±0.39 b	7.51±0.20 a	13.58±0.15 b	0.86±0.05 a	0.55±0.27 a
2020	N+PK	11.39±0.33 a	7.27±0.10 a	17.11±1.95 a	0.93±0.07 a	0.69±0.07 a
	0.75N+PK	12.00±1.19 a	7.28±0.14 a	17.01±1.77 a	0.88±0.21 a	0.63±0.10 a
	0.5N+PK	9.88±0.84 b	7.27±0.11 a	12.45±0.16 b	0.86±0.01 ab	0.67±0.07 a
	PK	9.84±0.44 b	7.48±0.16 a	11.76±0.82 b	0.81±0.03 b	0.59±0.05 a
2021	N+PK	11.41±0.05 ab	7.25±0.21 a	21.08±1.82 a	1.37±0.11 a	0.67±0.05 a
	0.75N+PK	11.65±0.06 a	7.28±0.14 a	20.95±1.27 a	1.27±0.02 a	0.66±0.19 a
	0.5N+PK	10.08±0.08 bc	7.25±0.02 a	14.01±2.01 b	1.15±0.10 a	0.49±0.10 b
	PK	8.89±0.13 c	7.39±0.10 a	13.33±1.18 b	1.10±0.28 a	0.47±0.08 b

The results show means ± standard deviations (n = 3). Different lowercase letters after values indicate significant differences between each treatment in the same year, $P < 0.05$. SOC, soil organic carbon; N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.

Table S2 The basic chemical properties of initial and treated straw under different N input levels after straw return

Treatment	Straw C (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})
Initial	**485.77±25.21 a**	**6.72±0.36 c**	**2.01±0.12 a**	**21.00±0.13 a**
N+PK	**428.86±17.82 b**	**17.20±0.51 ab**	**1.36±0.11 b**	**1.48±0.16 bc**
0.75N+PK	**429.00±30.21 b**	**16.72±0.45 b**	**1.45±0.10 b**	**1.18±0.14 c**
0.5N+PK	**427.72±29.96 b**	**18.15±1.03 a**	**1.81±0.17 a**	**1.86±0.10 b**
PK	**446.36±2.42 b**	**18.33±0.53 a**	**1.88±0.35 a**	**1.77±0.46 b**

The results show means ± standard deviations (n = 3). Different lowercase letters after values indicate significant differences between each treatment, $P < 0.05$. SOC, soil organic carbon; N, nitrogen;P, phosphorus; K, potassium; N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.

Table S3 The abundances of fungal and bacterial abundances across different N fertilizer level treatments after straw return

Treatment	Fungi abundance ($\times 10^7$ copies g^{-1} soil)	Bacteria abundance ($\times 10^7$copies g^{-1} soil)	Fungi: Bacteria ratio
N+PK	**0.63±0.16 bc**	**3.15±0.30 a**	**0.20±0.04 b**
0.75N+PK	**0.85±0.09 a**	**2.88±0.24 ab**	**0.30±0.05 a**
0.5N+PK	**0.57±0.04 c**	**2.87±0.42 ab**	**0.20±0.03 b**
PK	**0.39±0.05 d**	**2.17±0.43 b**	**0.18±0.02 c**

The results show means ± standard deviations (n = 3). Different lowercase letters after values indicate significant differences between each treatment, $P < 0.05$. N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.

Table S4 The fungal and bacterial alpha diversity under different N input levels after straw return

	Treatment	Chao1	Richness
Fungi	N+PK	1118.60±71.84 a	883.00±38.57 a
	0.75N+PK	1117.82±67.17 a	796.33±28.45 ab
	0.5N+PK	1063.37±84.82 a	781.00±33.87 ab
	PK	1054.50±22.29 a	772.33±27.54 b
Bacteria	N+PK	5917.52±149.48 a	4475.00±87.11 a
	0.75N+PK	5920.19±197.47 a	4396.67±27.43 a
	0.5N+PK	5881.07±152.30 a	4398.33±32.35 a
	PK	5672.76±82.25 a	4241.00±64.55 b

The results show means ± standard deviations (n = 3). Different lowercase letters after values indicate significant differences between each treatment, $P < 0.05$. N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.

Table S5 The relative abundances of keystone taxa across different N fertilizer level treatments after straw return

Treatment	FOTU22 (Module 3)	BOTU21 (Module 1)	BOTU6346 (Module 3)	BOTU240 (Module 2)
N+PK	**388.33**	**453.67**	**63.67**	**44.33**
0.75N+PK	**536.67**	**638**	**147**	**79**
0.50N+PK	**367**	**303.33**	**150**	**82.66**
PK	**109.33**	**421**	**18.33**	**63**

The results show means (n = 3). Different lowercase letters after values indicate significant differences between each treatment, $P < 0.05$. N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer. FOTU, the OTU in fungi; BOTU, the OTU in Bacteria.